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EVOKED RESPONSES TO ELECTRICAL STIMULATION IN THE COLONIAL HYDROID *CLAVA SQUAMATA*: A CONTRACTION PULSE SYSTEM [1]

DARRELL. R. STOKES AND NORMAN B. RUSHFORTH

Department of Biology, Emory University, Atlanta, Georgia 30322; Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106; and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Electrical activity has been recorded from several hydrozoans (phylum Cnidaria) representing two distinct classes of polyps—those which produce recurring behavioral events of spontaneous origin, for example, *Corymorpha* (Ball, 1973; Ball and Case, 1973); *Hydra* (Josephson, 1967; Josephson and Macklin, 1969; Passano and McCullough, 1962, 1963, 1964, 1965; Rushforth, 1966, 1971; Rushforth and Burke, 1971); *Millepora* (deKruijf, 1976); *Obelia* (Morin and Cooke, 1971a, b, c); *Tubularia* (deKruijf, 1977; Josephson, 1962, 1965, 1974; Josephson and Mackie, 1965; Josephson and Rushforth, 1973; Josephson and Uhrich, 1969); and those which are behaviorally quiescent, *e.g. Cordylophora* (Josephson, 1961b; Mackie, 1968); *Hydractinia* (Stokes, 1974a, b) and *Proboscidactyla* (Spencer, 1974). Despite these obvious behavioral differences, a review of these studies (Rushforth and Stokes, 1978) showed that there is at least one element of electrically evoked activity which is common to all species of both classes. These common elements are the large (0.5–15 mV), slow conducting (2–20 cm/sec), long duration (20–500 msec) potentials which are correlated with contraction of whole or isolated parts of a polyp. Examples include the Josephson Pulses (JPs) of *Cordylophora*, Hydranth Pulses (HPs) and Neck Pulses (NPs) of *Tubularia*, Stalk Pulses (SPs) and Hydranth Pulses (HPs) of *Corymorpha*, Contraction Pulses (CPs) or Contraction Bursts (CBs) of *Hydra*, Symmetrical Contraction Pulses (SCPs) of *Hydractinia*. Contraction Pulses (CPs) of *Millepora*, Contraction Pulses (KPs) of *Obelia* and the Tentacle Contraction Pulses (TCPs) and Colonial Pulses (CPs) of *Proboscidactyla*.

It has been proposed (Rushforth and Stokes, 1978) that these pulses are homologous representations of a fundamental conducting system termed the 'Contraction Pulse System' (CPS). This system functions to activate muscles of widespread distribution, or isolated blocks of muscles. In an effort to strengthen this proposal, we have investigated the evoked responses of the colonial gymnoblastic hydroid, *Clava squamata*. The purpose of the present study was to examine the electrical activity of this behaviorally quiescent species.

Materials and Methods

Collection and maintenance of animals

The Supply Department of the MBL, Woods Hole, provided all animals for this study. Only colonies growing on *Ascophyllum* were used. Colonies in which the

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polyps appeared unhealthy or damaged, or in which the alga had begun to decay, were discarded. All specimens were placed in an aerated, refrigerated (14° C) Instant Ocean System containing sea water, and were fed daily on newly hatched *Artemia* nauplii. Collected colonies were studied within 2 to 3 days. Glass microscope slide cultures of *Clava* were also made by dissecting two to three interconnected polyps from a colony and attaching them to the slide by fine surgical thread. Such cultures were maintained under conditions similar to those of the freshly collected colonies. New stolons grow out from the transplant, adhere to the glass slide and give rise to new polyps. After about 1 week the original transplant and the surgical thread can be removed, leaving only new growth attached to the slide.

Most experiments used whole colonies on their algal substrate. The alga was trimmed to about 2 to 3 cm length and pinned to the Sylgard bottom of a dish containing 500 ml of sea water. The temperature of the sea water in the dish was also maintained at 14° C by an outer jacket of circulating, refrigerated water. The attached hydroids did not appear to be damaged by this process and survived for several days under such conditions, when appropriately fed. In certain experiments single polyps were used. These polyps were excised from the colony by means of iridectomy scissors at the junction between polyp and stolon.

Stimulation and recording techniques

Suction electrodes were used for recording and stimulation. Plastic Tygon or polyethylene tubing was flame heated and drawn to diameters appropriate for attachment to a specific region of the polyp or stolon, usually 50 to 100 μm. The tubing was then squarely and evenly cut to insure minimal damage to the soft tissue. Glass electrodes modeled after those described by Josephson (1967) were used in experiments on isolated polyps. These electrodes have a bell-shaped tip in which the polyp can be held without damaging the tissues. Both kinds of electrodes can be attached by means of mild suction obtained by manipulation of a hypodermic syringe at the opposite end of the tubing or glass. Micromanipulators served to facilitate movement and placement of the electrodes. Both stimulation or recording could be achieved through these suction electrodes, and in either case, the indifferent electrode was a coil of chlorided silver wire placed in the experimental dish. Electrical stimuli were single or repetitive pulses delivered through a stimulus isolation unit. Conventional capacitor coupled amplifiers and display devices were used.

Results

General organization of the colony

Clava may be found at Woods Hole, Massachusetts in association with an intertidal brown alga, *Ascophyllum*. It is generally located at the branch points of the alga or within damaged flotation sacs, and only rarely along the lengthy exposed parts of the stem. Occasionally one can find *Clava* on rocks or wharf pilings. Colonies growing on *Ascophyllum* are comprised of upwards of 50 to 100 monomorphic polyps; the hydranth of each bears a naked hypostome and terminal

mouth, and some 20 to 30 filiform tentacles. The tentacles are uniformly distributed over the hypostomal region and do not form a distinct ring or rings. The lengthy stalk region below the hypostome is naked in immature polyps. Gonophores bud off from this stalk region 1 to 2 mm below the most proximal tentacles in mature polyps. The polyps vary in length, from about 0.5 to 1.5 cm, and are joined basally by a coenosarc of interconnecting stolons. The sexes are separate and a colony is comprised of all male or all female polyps, probably originating from a single planula. Occasionally, as reported by Föyn (1927), male and female colonies will occur together in the same clump.

General behavior and responses to mechanical stimuli

Spontaneous and rhythmically recurring behavior of individual polyps or groups of polyps does not occur under uniform conditions of illumination in *Clava*. Polyps may bend slowly or reorient individual tentacles, but the general observation is prevailing quiescence. Endogenous pacemaker systems like those responsible for the rhythmic behavior of *Tubularia* (Josephson, 1962; Josephson and Mackie, 1965) or *Hydra* (Passano and McCullough, 1962, 1965) do not appear to be present in *Clava*.

Mechanical stimulation of a polyp may induce weak or vigorous polyp activity depending on the intensity of the stimulus. Pinching an individual tentacle can result in contraction of that tentacle alone; contraction of the tentacle and movement of the hydranth towards the stimulated side; or contraction of the entire polyp. Contraction of the polyp may be graded, but in most cases, particularly colonies growing on *Ascophyllum*, it was not observed to spread to neighboring polyps. In rare cases, vigorous pinching of the hydranth resulted in a vigorous contraction of the polyp, and that contraction spread in a graded fashion to the neighboring polyps. Pinching a stolon also can elicit the same response; however, in no case were more than three polyps involved in this coordinated response.

Long-term electrical recordings from individual polyps show that electrical activity does not occur in *Clava* in the absence of behavioral events. Furthermore, many complex behaviors, particularly those associated with feeding, occur in the complete absence of detectable electrical events. Other complex responses induced by certain amino acids are associated with the generation of electrical potentials; however their role in feeding, if any, is not known.

Following mechanical stimulation of a *Clava* polyp, either single or multiple pulses can be recorded with a short latency (less than 400 msec) which correlate with the visual observation of overall polyp contraction. Weak pinching of any part of a polyp produces single electrical pulses with each contraction, whereas vigorous stimulation produces multiple electrical pulses and a more vigorous polyp contraction. These same results can be obtained by mechanical stimulation of the interconnecting stolons.

Electrical stimulation-correlated behavior and electrical activity

The electrical responses in this study have been recorded extracellularly from rather large blocks of tissue comprised of ectoderm, mesoglea and endoderm.

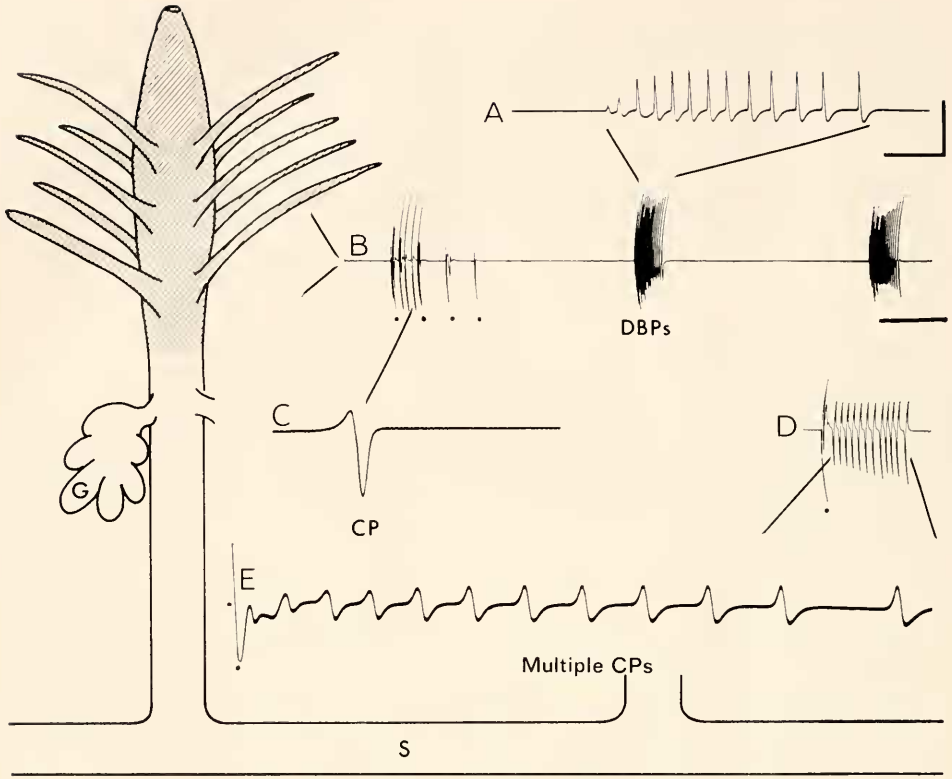

FIGURE 1. *Clava* polyp schematic showing representative kinds of evoked electrical pulses. A, delayed burst potentials similar to those shown in record B, but recorded at faster time base. Stimulus not indicated. B, a sequence of CPs and two bursts of DBPs evoked by four stimuli (dots beneath recording) applied to the burst generator region (hatched region). C, a typical CP evoked by stimulation of any part of the body column or tentacles. D, typical record of multiple CPs recorded from the polyp stalk. E, multiple CPs similar to those recorded in D, but at a faster time base. Dot indicates stimulus artifact. G, gonophores, S, stolon; Vertical scale A applies also to B, C, D, E, =0.5 mV; Horizontal Scale A applies also to C, E, =0.6, 0.2, 0.3 secs, respectively; Horizontal Scale B applies also to D =5.0 sec.

Positive identification of the underlying morphological source of electrical signals generated by these tissues is difficult and hence, for the moment, we cannot identify these events as neural, muscular, or epithelial. Instead the neutral term "conducting system" (Josephson and Mackie, 1965) will be used to define the substrates of these electrical events.

Two distinct kinds of electrical activity can be recorded from *Clava* depending on the stimulus site. Evidence presented here and in a subsequent report (Rushforth and Stokes, in preparation) indicates that these two events represent activity in separate conducting systems. One of these conducting systems is activated only by direct stimulation of the hydranth distal to the gonophore stalks (Fig. 1, hatched region). The electrical events (Figs. 1A, B) occur as a burst or a

TABLE I

Characteristics of electrically stimulated contraction pulses in Clava: *mean ± s.e.*

Pulse type	Latency (msec)	Interpulse interval (msec)	Pulse duration			Pulse amplitude mV	Conduction velocity cm/sec
			Rise time (msec)	+ve to −ve Peak (msec)	Total (msec)		
A. Single contraction pulses	331 ± 109 (3)*	—	74 ± 26 (2)	48 ± 11 (4)	144 ± 20 (4)	0.53 ± 0.03 (4)	2.6 ± 0.2 (11)
B. Multiple contraction pulse bursts	330 ± 70 (2)	371 ± 19 (9)	73 ± 6 (2)	50 ± 7 (9)	140 ± 8 (9)	0.61 ± 0.10 (9)	2.1 ± 0.3 (5)

* Values in parentheses = N.

program of multiple bursts of pulses after a long latency ($\bar{x} = 19.2 \pm 1.4$ sec, $N = 18$) and are referred to as Delayed Burst Potentials (DBPs). A burst of DBPs on an expanded time scale is shown in Figure 1A. In no case have DBPs been initiated by stimulation of stolons or the stalk region proximal to the gonophores. DBPs correlate with a synchronized depression of the tentacles and, at least to the first few bursts of a program, with a symmetrical shortening of the body stalk. The stalk contraction element is not apparent after the first few bursts of a program. Also, the pulses within a burst appear much more homogeneous following loss of an observable stalk contraction. The conducting system underlying these bursts of pulses, called the Delayed Burst System (DBS) is the subject of a subsequent report (Rushforth and Stokes, in preparation).

A second type of electrical event can be evoked by stimulation of the polyp at any location—tentacles, hypostome, body column or base. Characteristically, these potentials are single (Figs. 1B, C) or multiple pulses (Figs. 1D, E), which are similar in all respects to those evoked by mechanical stimulation. These potentials

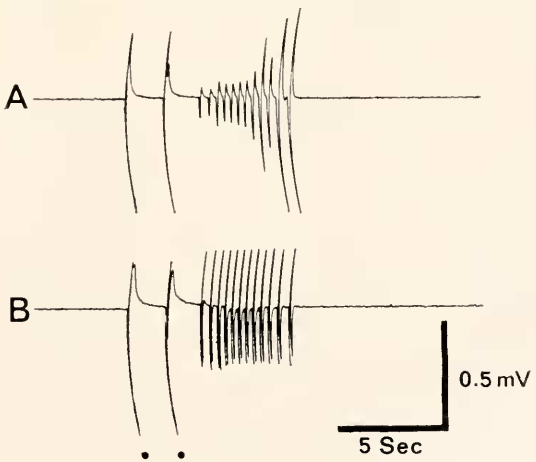

FIGURE 2. Facilitation of CP potentials within a "burst", recorded at two sites (A and B) on a single polyp stalk. Two stimuli were applied (dots) adjacent to site B.

are correlated with a symmetrical, often localized contraction of the polyp and are thus referred to as Contraction Pulses (CPs). The conducting system underlying the CPs is referred to as the Contraction Pulse System (CPS). The threshold for activation of the CPS is generally below that for the DBS. Both single and multiple CPs appear to originate within the tissues beneath the stimulating electrode and have a short latency (about 300 msec) in comparison to the DBPs. Characteristics of the electrical pulses associated with single and multiple CPs are presented in Table I. In summary, both single CP events and individual CPs of a multiple sequence are nearly identical with respect to pulse rise time (75 msec), positive to negative peak (50 msec), total pulse duration (140 msec) and pulse amplitude (0.5–0.6 mV). The refractory period for single CPs was determined to be about 200 to 250 msec, and the interpulse intervals for multiple CPs are on the average about 370 msec. As shown in Figure 1E, the interpulse intervals tend to elongate towards the end of a CP burst. CPs are generally biphasic, although triphasic CPs have also been recorded, usually following the first stimulus of a regime of stimuli given to a fully expanded polyp.

Both single and multiple CPs are nonpolarized conducted events (Table I). The conduction velocity was determined by recording from two Tygon suction electrodes attached to the same side of the poylp while stimulating through a glass holding electrode. From these records, the time delay between negative peaks of the electrical event recorded at the two sites and the measured distance between the recording electrodes were used to compute the conduction velocity. The mean conduction velocity for single CPs is 2.6 cm/sec ($N = 11$) and for multiple CPs 2.1 cm/sec ($N = 3$). For three animals, conduction velocities for single CPs were determined for both the proximal and distal directions. No significant differences were observed ($\bar{x} = 2.3 \pm 0.1$ distally and $\bar{x} = 2.2 \pm 0.3$ proximally). The mean conduction velocity for DBPs is 9.8 cm/sec ($N = 7$).

The responses of a single polyp to electrical stimulation appear to be graded with stimulus intensity. A single threshold shock to a tentacle may result only in contraction of the stimulated tentacle. A small CP is associated with the tentacle contraction. Similarly, threshold stimulation of the hydranth may result only in contraction of the hydranth region, with associated CPs, and in no apparent contraction of the stalk region. Responses of the polyp are more extensive and also more vigorous with increasing numbers and intensity of stimuli. The amplitude and number of recorded CPs decrement with distance from the stimulus site. Multiple CPs of large amplitude occur in regions of vigorous contraction, the number and amplitude diminishing in regions where contraction intensity is also reduced. During repetitive stimulation, contraction spreads to and involves a greater part of the polyp, an observation which appears to correlate with facilitation of CPs to each shock. Single threshold shocks generally give a localized polyp contraction and a single CP. Multiple shocks of threshold intensity or single suprathreshold stimulation of either proximal or distal regions of the polyp stalk can evoke multiple firing of the CP system. Some 30 or more CPs have been recorded to a single suprathreshold stimulus. Multiple firing of the CP system such as that shown in Figures 1D, E is correlated with a prolonged, continuous shortening of the polyp stalk, which often reduces the polyp to a stubby ball. In

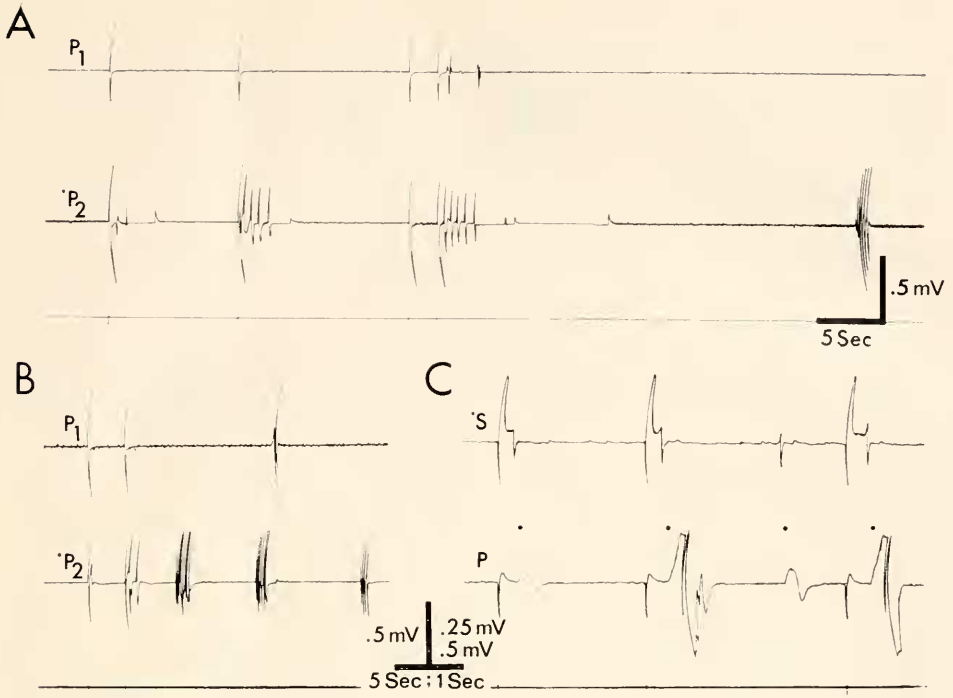

FIGURE 3. Interpolyp communication. A, simultaneous recordings from two interconnected polyps (P_1 and P_2) following application of four stimuli to one of them (asterisk). Largest events (stimulus artifacts) correlated to stimulus record (lower channel). Note that a burst (DBPs) occurs only in the stimulated polyp. B, same as A, but note that a CP occurs in P_1 shortly after a burst of DBPs in P_2. C, simultaneous recordings from a polyp (P) and an attached stolon (S) while stimulating the same stolon (asterisk). Note the small potentials in S correlate with repetitive CPs (dots) in P which facilitate to each stimulus (marked in lower trace).

this condition, the polyp is refractory to further stimulation, and it may take 30 min or more before the polyp fully expands once again.

Dual recordings from a single polyp during multiple CP firing (Fig. 2) also show more vigorous contraction adjacent to the stimulus site and uniformly large CPs (Fig. 2B), while at a distant recording site the contraction may not be apparent initially but becomes increasing more vigorous throughout a CP burst. The CPs in such a burst show a marked facilitation (Fig. 2A). As a wave of contraction passes a recording site, subsequent stimulation can result in a marked reduction and even a defacilitation of CPs within a burst.

Stimulation of the hydranth region of a polyp, as shown in Figure 1B, can evoke both single and multiple CPs in addition to DBPs. In this record, the first of four stimuli initiated some four CPs and the third stimulus a single CP of smaller amplitude. And finally, some 12 sec after the fourth stimulus, the DBS fired a burst of potentials.

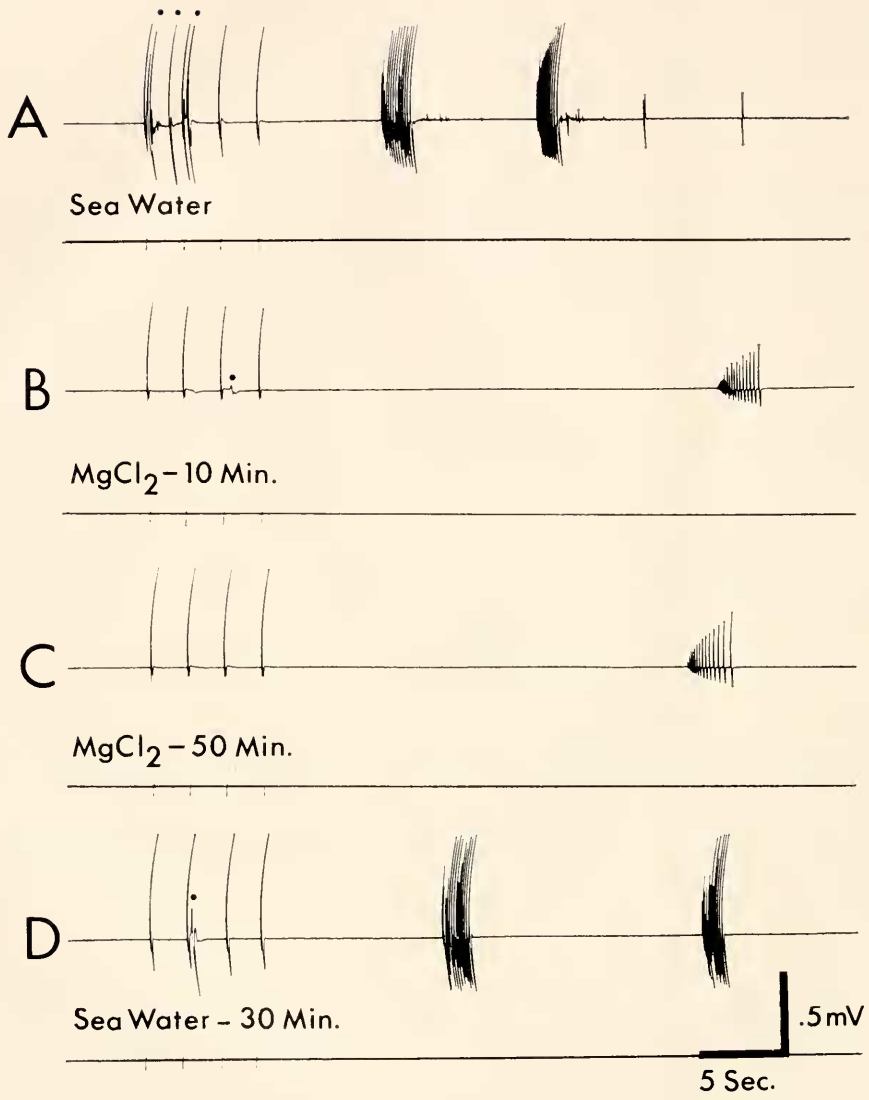

FIGURE 4. Effects of $MgCl_2$. A, recording from an isolated polyp in sea water. Stimuli in this and following records are marked in lower trace. B, same as A, after 10 min in $MgCl_2$. C, same as A, after 50 min in $MgCl_2$. D, same as C, following return of polyp to normal sea water for 30 min.

Interpolyp communication

Communication from polyp to polyp by means of the interconnecting network of stolons has been difficult to demonstrate in wild colonies. In such colonies, the polyps arise from a tangled mass of stolons which makes it difficult to determine

which of the polyps are directly connected. In addition there are numerous symbionts (crustaceans, platyhelminths, protozoans) which live in association with the tangled stolons, many of which have been observed to feed on the soft tissues of the colony. It seems likely that these symbionts disrupt the structural organization of the colony, leaving whole portions of the colony or even individuals functionally isolated from each other. In only a few colonies did mechanical stimulation of one polyp affect the behavior of adjacent polyps. These were young colonies with new growth and no apparent damage to the stolons joining the two polyps. In all cases, very intense mechanical stimulation was necessary to show spread of excitation to an adjoining polyp, and in all cases only two polyps were involved.

Slide culture colonies provide a better preparation for the study of interpolyp communication. The stolons grow out in rather straight formations and give rise to polyps at intervals of about 3 to 5 mm. One can maintain cultures free of symbionts and also easily observe the integrity of the stolon network. Recording from and stimulating stolons and polyps which arise from them is greatly facilitated in culture colonies, and consequently it is easier to demonstrate interpolyp communication. Three examples of such are shown in Figure 3. In record A, recording electrodes were placed on two polyps in such a culture colony. One polyp, P_2, was stimulated with four shocks in the region on the burst generator. CPs were recorded first in P_2 (the polyp stimulated), which eventually, after the fourth stimulus, were conducted through the stolon to P_1 where two small CPs were recorded. P_2 was observed to contract following the first stimulus, P_1 not until after the fourth stimulus. The DBS was activated in P_2 as can be seen by the delayed burst in this record, but the DBPs did not conduct through the stolons to the distant polyp (P_1). In Figure 3B, the polyp stimulated (P_2) gave rise to CPs and DBPs, the second burst of DBPs appears to activate the CP system of the distant polyp. While this is not conclusive evidence for an interaction between the DBS and CPS, it is supportive of additional evidence for such an interaction presented subsequently (Rushforth and Stokes, in preparation). There is no evidence that CPs initiate activity in the DBS.

In Figure 3C, recording electrodes were placed on a stolon (S) and a polyp (P) arising from that same stolon. The stolon was stimulated with three stimuli all of which initiated a small (150 μV), fast spike in the stolon which correlated with CPs in the distant polyp. These CPs facilitated to each stimulus. In one case the small spike fired independently of an electrical stimulus, and it too correlated with a CP in the polyp and contraction of the polyp. DBPs were never initiated by stimulation of the stolon. Repetitive stimulation of a stolon results in a graded spread of excitation involving only the CP system. Spread of excitation to some 8 to 10 interconnected polyps in a colony has been observed, polyps closer to the stimulus site showing more CP activity than those more distant.

Effects of $MgCl_2$

The effects of isosmotic $MgCl_2$ on the mechanical and electrical activity of attached individual polyps are shown in Figure 4. Electrical activity and the visually monitored behavioral responses were first studied in normal sea water and when typically consistent responses were observed, isosmotic $MgCl_2$ was

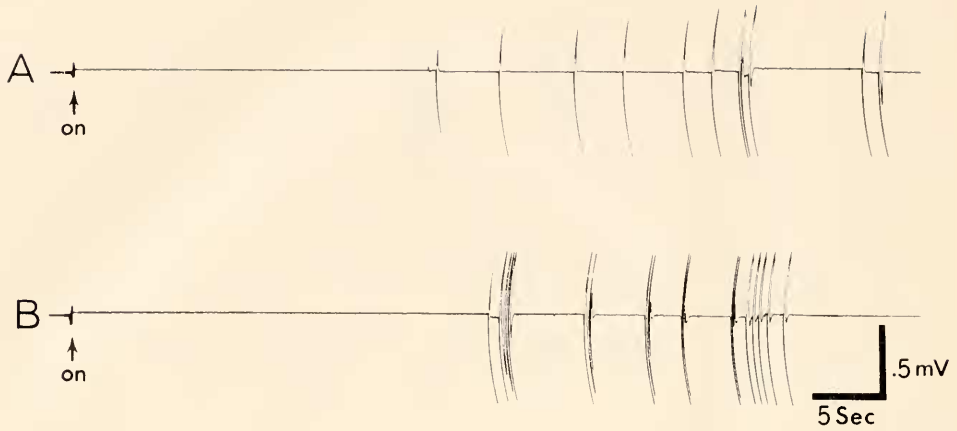

FIGURE 5. Light induced CP activity (A) and multiple CP activity (B) of *Clava* following a 15 min period of dark-adaptation. Onset of illumination is indicated by the arrows.

added to the experimental dish until a final concentration of 40% was obtained. The polyps were stimulated in the burst generator region with four shocks at 2-second intervals; the stimulus burst being repeated at 10-minute intervals. Only polyps giving both CPs and DBPs to two consecutive stimulus tests 10 minutes apart in normal sea water were used. A typical record in normal sea water is shown in Figure 4A. Both visual and electrical records were made following each stimulus regime. The results were similar for five polyps, one from each of five colonies.

Isosmotic $MgCl_2$ abolishes nearly all CP activity as well as the correlated behavioral responses after ten minutes exposure (Fig. 4B). The DBPs, however, persist in somewhat altered form and increased latency, despite the absence of a behavioral response. The bursts appear to consist of uniformly facilitating pulses of somewhat diminished amplitude from that in normal sea water. Delayed bursts have been recorded for up to 3 hours in $MgCl_2$ without further change. Both CPs and typical DBP bursts are restored following exposure to normal sea water for a brief period (10–30 min; Fig. 4D).

Effects of light

Whole colonies of *Clava* respond to sudden sharp increases in illumination after an apparent delay of some several seconds. Polyps contract symmetrically and often in distinct steps in much the same manner as occurs following CP activity. There does not appear to be a systematic co-ordination of polyp contraction throughout the colony; polyps nearest the source of illumination generally contract first, but there is no set order of responses. Synchronized tentacle depression characteristic of DBS activity has not been observed. The latency of the contraction responses coupled with the absence of tentacle depression led to a series of experiments designed to determine whether electrical events result in CP activity, DBS activity, or perhaps an as yet undescribed conducting system.

TABLE II

Responses of dark-adapted Clava *polyps to the onset of illumination. means ± s.e. (N = 5).*

	Number of pulses			Latency* to first pulse
	Before	During	After	
a) Intact polyp Midstalk	0.3 ± 0.2	10.9 ± 2.7	0.6 ± 0.3	35.4 ± 4.8
b) Intact polyp Hydranth	0.8 ± 0.8	13.5 ± 2.4	1.8 ± 1.8	23.4 ± 5.8
Prox. stalk	1.0 ± 0.7	12.1 ± 2.0	3.1 ± 2.7	24.9 ± 7.0
c) Transected polyp Hydranth	0.5 ± 0.5	6.3 ± 4.0	0.6 ± 0.6	10.2 ± 1.2
Prox. stalk	0.6 ± 0.2	11.8 ± 0.7	0.8 ± 0.4	30.4 ± 6.4

* Defined as the time interval in seconds from the onset of illumination to the intitation of electrical activity.

Recordings were made from single *Clava* polyps of a whole colony which had been dark-adapted for 15 min. The results of these experiments showed that polyps are quite sensitive to light, producing both single (Fig. 5A) and multiple (Fig. 5B) electrical pulses during the initial stages of stimulation. The numbers of pulses recorded from single (midstalk) and dual sites (hydranth and proximal stalk) were determined for a control period of 2 min before light stimulation, and a 2-min period following light stimulation. The light source for all experiments was a 6-V bulb from a microscope lamp set about 15 cm from the preparation. Light intensity was similar throughout. The experimental regime was repeated for a mimimum of five trials with a 15-min period of dark-adaptation between successive light exposures, for five polyps. The results for single recordings for all experiments are shown in Table II. The mean number of pulses recorded during the 2-minute light exposures for all experiments were on the order of 10 to 40 times greater than either before or after the light stimulation period. The mean latencies (defined as the time interval from the onset of illumination to the onset of the electrical response) varied from about 10 sec in the isolated hydranth preparation to 35 sec in stalk recordings. Although most of the electrical activity appears in the form of single or doublets of pulses, they are often produced in distinct groups. The mean number of pulses per group is significantly different for the hydranth and proximal stalk regions (5.1 ± 0.8 and 2.0 ± 0.1, respectively). An intermediate value of 3.4 ± 0.1 was obtained from midstalk recordings.

Electrical responses to the onset of illumination have been recorded also from isolated tentacles (three of five preparations) isolated hypostomes (three of five preparations) and hydranth preparations from which all tentacles and the hypostome were removed (six of six preparations). We have not examined isolated pieces of stolon.

Addition of $MgCl_2$ abolishes the characteristic responses to the onset of illumination within a period of five minutes. The only exception occurred in a single isolated hypostomal preparation which produced DBPs that persisted in somewhat modified form for three hr, with no observable behavioral correlate.

Discussion

Evidence from this study indicates that the CP system of *Clava* is another example of a fundamental muscle activating conducting system found in hydrozoan polyps. Electrical potentials from this system are correlated with symmetrical contractions of whole, or regions of whole, polyps, and are similar in pulse characteristics and conduction velocity to the electrical potentials correlated with contractions in other hydroids. Such pulses have the common features of long duration (up to 500 msec), relatively large amplitude (up to 15 mV), and slow conduction velocity (2–21 cm/sec) when recorded externally (Rushforth and Stokes, 1978).

Activation of CPs in *Clava* results always in some degree of symmetrical shortening of the polyp in the direction of the interconnecting network of stolons. The polyp may shorten in one or more steps depending upon the intensity and frequency of stimulation. Following successive contractions, the polyp is reduced to a stubby ball. Furthermore, when contracted, the polyps are shielded between the branches or within the damaged flotation sacs of the alga upon which they naturally occur. These responses are adaptive in that they provide a limited degree of protection for the exposed, softbodied polyp from potentially hazardous environmental stimuli. Similar protective functions of polyp contraction are apparent also in *Hydractinia* where the polyps ultimately contract below a layer of chitinous spines (Stokes, 1974b); in *Millepora*, where the polyp contracts into a calcified skeleton (deKruijf, 1976; and in *Obelia* where the polyp withdraws into a hydrotheca (Morin and Cooke, 1971a). Protective withdrawal, like the escape responses of insects and crustaceans with giant fiber systems, may be the major role of the CP system in *Clava*. Behavioral activities such as those associated with prey capture, feeding, and defecation are more complex and are probably integrated by other conducting systems within the polyp. Multiple conducting systems have been physiologically identified in all hydroid polyps thus far examined, though in no case is the behavior totally attributable to known conducting systems. *Clava* has at least two distinct conducting systems within the polyp. In addition to the CPS, a non-polarized Delayed Burst System (DBS) has been identified which produces programs consisting of bursts of pulses. Such programs are initiated after a long delay (about 20 sec) following stimulation. The DBS can be distinguished from the CPS by its somewhat higher threshold of activation; resistance to Mg^{2+}; restricted location within the hydranth of the polyp; and different behavioral correlates (tentacle depression vs. polyp contraction). The potentials produced by the DBS also have different conduction velocity and pulse characteristics. DBPs have shorter duration (50–60 msec), shorter interpulse intervals (170–230 msec), and faster conduction (8–12 cm/sec) than multiple contraction pulses (*cf.* Table I).

Though the functional significance of the DBS is not known, there is evidence to suggest that it interacts with the CPS. Tentacle depression together with polyp contraction is observed during the initial delayed bursts following DBS activation. These initial delayed bursts often appear to contain CPs interspersed with the DBPs (Stokes and Rushforth, personal observations). The CP elements in the delayed bursts are absent in the latter phase of a long program of bursts.

when the polyp is reduced to a stubby ball and contractions are no longer apparent. In this contracted state the CP system is not evoked by electrical stimulation. In addition, exposure of a polyp to Mg^{2+} eliminates contractions, and results in delayed bursts which appear to consist of a single pulse type, presumably DBPs. These observations suggest that the CP system is excited initially by the DBS and polyp contraction is caused in the early phase of a burst program. However, the system becomes increasingly refractory and CPs drop out in the final stages of the program.

Simultaneous recordings from two polyps connected by a stolon support the hypothesis that the DBS excites the CPS. Activation of the DBS by stimulation of the hydranth of one polyp can trigger CPs in the same polyp which then give rise to a CP in a neighboring second polyp. Very small potentials recorded in the muscle-free stolons interconnecting the two polyps correlate with the observed polyp contractions. These pulses may represent activity in nerve cells of an interconnecting nerve net. Nerve cells have been identified in the stolons of *Hydractinia* (Stokes, 1974a) but have not yet been looked for in *Clava* stolons. Single or multiple CPs of one polyp can also serve to initiate CPS activity in a second interconnected polyp. However, we have observed no case of CP triggering of DBPs. Interactions of the DBS and CPS in individual polyps and interactions of the CPS from polyp to polyp provide for a means of colonial co-ordination. Föyn (1927) was able to identify members of individual, interspersed colonies of *Clava* by pinching one polyp and observing which additional polyps contracted. Co-ordinated responses of polyps comprising a colony have been observed also in *Cordylophora* (Josephson, 1961b) and *Hydractinia* (Josephson, 1961a; Stokes, 1974b) where they are presumed to be protective and co-ordinated by conducting systems underlying muscle contraction. Clearly all members of the colony would be served by advanced notice of a predator attempting to feed on one member of the colony.

The electrical activity evoked by dark-adapted *Clava* polyps which occurs after the onset of illumination has certain features of both DBS and CPS activation. The recorded latencies following the onset of illumination to the initiation of electrical activity are usually quite long, sometimes on the order of 35 sec. The latencies of DBPs evoked by electrical stimulation are often equally as long. However, despite such long latencies, the following evidence suggests that light activates the CPS; removal of the burst generator region by transection of a polyp well below the hydranth does not affect the generation of pulses in the remaining proximal stalk region, clearly demonstrating that the burst generator region is not necessary for the light induced responses. Though patterns of light-induced potentials sometimes consist of programs of burst, such bursts are closer in pulse characteristics to multiple CPs than DBPs. Frequently the light response consists of sets of widely spaced single or double pulses; Mg^{2+} abolishes the light-induced activity and the behavioral responses in the same time course as CPs.

The long latency of the photic responses in *Clava* is similar to that recorded for *Hydractinia* (21–70 sec; Stokes, 1972). Such long latencies may result from a similar mechanism of pulse generation and spread of excitation. In fact, it is a contraction pulse system (the SCP) which is activated by light in *Hydractinia*. The latency of the response may reflect the levels of photosensitive pigments which

have accumulated during the period of dark adaptation (Ballard, 1942). In *Clava* we have preliminary data showing that there is an inverse relationship between the length of the dark adaptation period and the latency to the response. The light receptors, be they photosensitive pigments or some as yet unidentified photoreceptor, would appear to be widespread throughout a polyp. However, since more pulses are induced in the hypostomal region, than in the body column and base of the polyp, there may be relatively more pigment or more photoreceptors in this region. In *Hydractinia* electrical activity occurs only by direct photic stimulation of the basal mat (Stokes, 1972). We have not examined the light sensitivity of *Clava* stolons.

Very little is known of the morphological substrates of the CPS or DBS in *Clava*. Preliminary histological studies utilizing reduced methylene blue show the presence of nerve cells in the stalk region. The burst generator region and the stolons remain to be examined. However, electrical recordings suggest that CPs are not solely a result of neuronal activity. The electrical potentials are too large and of too long in duration to originate from the small nerve cells. Furthermore, they are conducted much more slowly than one would expect for purely neuronal pathways. It has been suggested that similar large potentials from other hydroids are propagated in epithelial sheets via low resistance junctional specializations (Josephson, 1967). Septate junctions have been found connecting epitheliomuscular cells of *Hydra* (Wood, 1959) and *Hydractinia* (Stokes, 1974a) where they have been implicated in contraction responses. Josephson and Macklin (1967) have shown that the CP of *Hydra* is a transepithelial event. On the other hand, as Mackie (1970) suggests, conduction of such large potentials may combine both neuronal and epithelial elements.

In this study we have shown that the colonial hydroid, *Clava squamata* possesses a Contraction Pulse System whose properties are similar to the CP systems of other hydroids. It provides further evidence that the CP system is a common conducting system in hydroid polyps. Studies with other hydrozoans should indicate whether it is a universal feature.

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Summary

1. At least two conducting systems are present in the colonial hydroid, *Clava squamata,* the contraction pulse system (CP system) which initiates symmetrical polyp contraction, and a delayed burst system (DBS) which is correlated with tentacle depression and polyp contraction.

2. The CP system has properties similar to contraction pulse systems of other hydroids; its electrical pulses are of large amplitude (greater than 0.5 mV) and long duration (150 msec), and slow conduction velocity (2–3 cm/sec).

3. The CP system courses through the polyps and their interconnecting stolons.

Electrical stimulation of a single polyp gives rise to CPs associated with contraction of that polyp, which sometimes can be recorded also in adjacent polyps.

4. Isosmotic $MgCl_2$ abolishes CPs and associated column contractions, but does not suppress delayed burst pulses.

5. Light initiates contractions of the polyp and correlated CPs.

6. It is postulated that the CP system of *Clava* is similar to contraction pulse systems previously described for other hydroids.

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